Arab Gulf J. scient. Res. **3** (2), pp. 735-744 (1985)

Isolation and Identification of Infectious Bovine Rhinotracheitis Virus in Saudi Arabia

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ABSTRACT. Infectious bovine rhinotracheitis (IBR) virus was isolated for the first time in Saudi Arabia from tracheal mucus collected from a dead cow during an outbreak of respiratory disease associated with anaerobic bacterial infection. Cytopathic effect was detected in calf kidney cell culture on the fourth day post inoculation. The virus isolate, designated Riyadh/5, was sensitive to ether and chloroform and was neutralized by immune serum prepared against a known strain of IBR virus. The possible relationships between infections with clostridia and IBR virus are discussed and the present status of infections with IBR virus in Saudi Arabia are reviewed.

Infectious bovine rhinotracheitis (IBR) is a disease of cattle caused by a herpesvirus which is distributed throughout the world (Straub 1978). It has been associated with many different clinical syndromes:

1) Diseases of the respiratory system and eye

a) Upper respiratory disease or classical IBR (Schroeder and Moys 1954, Madin *et al.* 1956).

b) Conjunctivitis and keratoconjunctivitis (Abinanti and Plummer 1961, Hughes *et al.* 1964).

c) Ocular carcinoma (Taylor and Hanks 1969).

2) Diseases of the reproductive system

a) Infectious pustular vulvovaginitis (IPV) in cows (Kendrick *et al.* 1958, Gillespie *et al.* 1959).

b) Infectious pustular balanoposthitis (IPB) of bulls (Studdert *et al.* 1964).

c) Infertility (Mare and van Rensburg 1961).

d) Abortion (McKercher and Wada 1964).

e) Mastitis (Gourlay *et al.* 1974, Roberts *et al.* 1974).

3) Diseases of the central nervous system

a) Meningo-encephalitis in calves (Johnston *et al.* 1964).

b) Encephalomyelitis (Beck 1975).

4) Diseases of the alimentary system

a) Enteritis of adult cattle (Wellemans *et al.* 1974).

b) Diarrhea associated with generalized fatal disease of calves (Baker *et al.* 1960).

5) Dermatitis (Bwangamoi and Kaminjolo 1971).

In addition, inapparent latent infections with IBR virus are not uncommon (McKercher *et al.* 1963). Such infections can be activated by stress factors and corticosteroids (Sheffy and Rodman 1973).

Neutralizing antibody against IBR virus was detected in many serum samples from Saudi cattle examined at the Central Veterinary Laboratories, Weybridge, United Kingdom, in 1980 (M. Lucas, personal communication) and at the Animal Virus Research Institute, Pirbright, United Kingdom, in 1981 (R.S. Hedger, personal communication). This report describes the first isolation of IBR virus in Saudi Arabia. The case was an adult cow which died during an outbreak of acute respiratory disease which appeared to be complicated by infection with *Clostridium* spp.

History of the Disease

The syndrome occurred in the milking herd of a farm near Riyadh. This herd consisted of 35 Holstein-Friesian cows imported from the United States in January 1982, 4 Holstein-Friesian cows imported from France in July 1981, 7 Zebu cows imported from Pakistan several years ago and 11 local cows. The animals were vaccinated against foot and mouth disease, rinderpest and hemorrhagic septicemia a few months before the appearance of this syndrome. Clinical symptoms and mortality were observed only among the Holstein-Friesian cows and their calves, while the Zebu and local cattle remained healthy. Another group of 30 Holstein-Friesian dry cows imported from the U.S.A. at the same time as the 35 imported

in January 1982 and housed about 200 meters away from the milking cows remained healthy.

Clinical symptoms appeared on May 31, 1982. Fever (41-42°C), anorexia, redness of nostrils, conjunctivitis, watery nasal and lacrimal discharge and accelerated respiration were present at first. By the second day, nasal and lacrimal discharges became purulent, small ulcers appeared in the nostrils and scabby crusts were observed on the muzzle. Petechial hemorrhages were present on the papillae of the buccal cavity. In some cases, lameness and severe diarrhea with dehydration also occurred. Subcutaneous emphysema in the shoulder and lumbar regions as well as swelling of the superficial lymph nodes were present in all sick cows. There was swelling and hyperemia of the vulva and in some cases, ulcers on the mucus membrane of the vagina were observed. There was red patches on the skin of the udder of one cow. Two cows aborted in the second and third months of pregnancy, respectively. The sick cows remained recumbent until death. Treatment with broad spectrum antibiotics or combinations of streptomycin and penicillin and attempts toward rehydration therapy with glucose and saline solutions were not effective. Of 12 affected cows, only one cow recovered; the others died between June 1 and June 10, 1982.

The lesions at necropsy were mainly gelatinous edema with gas pockets in the muscles behind the shoulder and in the hip region, subcutaneous emphysema, large amounts of brown fluid in the abdominal cavity, enlargement of the liver and gall bladder, slight inflamation and congestion of the small intestine, pneumonic areas and emphysema in the lungs and bloody froth in the trachea with hemorrhages in the tracheal mucosa. Lymph nodes were congested but the spleen appeared normal.

In calves, the clinical symptoms were fever (41°C), rhinitis, conjunctivitis, coughing, salivation, nasal and lacrimal discharges, accelerated respiration, severe diarrhea and lameness. Lesions included ulcers in the nostrils and buccal cavity, tracheitis, bronchopneumonia and gastroenteritis. No subcutaneous emphysema or gelatinous edema was observed in the calves. Affected calves did not respond to antibiotics and/or fluid replacement therapies. Of 21 Holstein-Friesian calves, 7 were clinically ill and all died.

Material and Methods

Collection of Samples

On June 4, 1982, nasal swab samples were collected from 3 milking cows and 2 calves with ulcers in the nostrils. Swabs were also used to collect vaginal mucus from two of the above 3 cows which were also showing ulcerative vulvovaginitis. Lymph nodes and tracheal mucus samples were also collected from another cow at necropsy. Each swab was placed in 3 ml of phosphate buffered saline containing

1000 units of penicillin and 1 mg dihydrostreptomycin per ml for transport to the laboratory. In addition, samples were collected for bacteriological examination. All samples were carried to the laboratory in a chilled thermos bottle.

Tissue Culture

Primary or secondary calf kidney (CK) cell cultures were prepared according to a standard technique (Madin *et al.* 1957). The growth medium consisted of Hank's balanced salt solution containing 0.5% lactalbumin hydrolysate, 0.0016% phenol red and 10% newborn calf serum. Antibiotics were added at a concentration of 100 units penicillin and 0.1 mg dihydrostreptomycin per ml of medium. For maintenance, Eagle's minimum essential medium supplemented with 0.5% lactalbumin hydrolysate and 2% horse serum was used.

Virus Isolation Procedures

The transport medium was centrifuged at 3000 rpm for 10 min. The supernatant fluids were used as the inocula. Lymph node tissues were homogenized in about 10 times their volume of phosphate buffered saline (pH 7.2) with 10 times concentration of antibiotics used before and centrifuged as described above. Each of 2 prewashed CK monolayer cultures grown in 50 ml Falcon bottles was inoculated with 0.2 ml of the supernatant fluid from each specimen. After absorption for 2 hr at 37°C, the cultures were washed, fresh medium added and the inoculated bottles incubated at 37°C. Microscopic examination for cytopathic effect (CPE) was done daily. Medium was changed every 3 days. Subculturing was usually done after 10 days, or whenever there was any indication of CPE.

Infectivity Assay

The infectivity titer of the virus isolate was determined by detecting CPE in CK cell culture monolayers grown in the wells of Microtiter plates inoculated with 10-fold dilutions of the virus as described by Hafez and Frey (1973). End points were calculated using the method of Reed and Muench (1938).

Sensitivity to Ether and Chloroform

The sensitivity of the virus isolate to ether was tested according to the techniques described by Andrewes and Horstmann (1949). Its sensitivity to chloroform was tested according to the method of Feldman and Wang (1961).

Serological Identification

Two lots of serial 10-fold dilutions of the virus isolate were mixed with equal volumes of 1:10 dilutions of the following sera:

1) Hyperimmune rabbit serum against the AH/248 strain of IBR virus (Hafez *et al.* 1976).

2) Fetal bovine serum (Flow Laboratories, Irvine, Scotland) as a negative control serum.

After incubation for 60 min at 37°C, 0.1 ml of every virus serum mixture was pipetted into each of 4 wells of a Microtiter plate; 0.05 ml of a CK cell suspension containing 10^5 cells per ml was added to each well. After 7 days incubation at 37°C, cells in each well were examined for CPE.

Results

Isolation

On the fourth day post infection (p.i.), CPE was observed in the cultures inoculated with sample number 5 (the tracheal mucus collected from the dead cow). Initially, foci of rounded cells appeared; these cells 'ballooned' and formed small refractile syncytia. As CPE progressed, strands of cytoplasm linking affected cells were seen. Complete lysis of the monolayer occurred on the *8th* day p.i. Upon subculture of this cytopathogenic agent (designated Riyadh/5) in CK cell cultures, CPE was detected on the *2nd* day p.i.

There was no CPE in cell cultures inoculated with other samples even after 2 blind passages.

Sensitivity to Ether and Chloroform

The infectivity of the Riyadh/5 virus isolate was completely lost after treatment with ether and chloroform (Table 1).

Serologic Identification

More than 99.999% (5 $\log_{10}$) of the virus infectivity was neutralized by treatment with a 1:10 dilution of hyperimmune serum prepared against a known strain

Table 1. Sensitivity of the Riyadh/5 virus isolate to ether and chloroform.

Virus material	Infectivity titer[1]
Before treatment	$10^{6.5}$
After treatment with ether	$<10^{0.5}$ (2)
After treatment with chloroform	$<10^{0.5}$ (2)

1. Log 10 $TCID_{50}$ per ml.
2. No cytopathic effect was observed in initial dilution of 10^{-1}.

Table 2. Neutralization of the Riyadh/5 virus isolate.

Serum[1]	Infectivity titer[2]
Hyperimmune serum against AH/248 strain of infectious bovine rhinotracheitis virus	$10^{1.0}$
Fetal bovine serum	$10^{6.23}$

1. 1:10 serum dilution mixed in equal amounts with serial virus dilutions.
2. Log 10 $TCID_{50}$ per ml.

of IBR virus. There was no loss of infectivity when the same virus preparation was treated with 1:10 dilution of fetal bovine serum (Table 2).

Discussion

The identification of the viruses causing diseases of cattle in Saudi Arabia is a first step towards their control. The neutralization of the Riyadh/5 virus isolate with reference immune serum prepared against a known IRR virus confirms that this is the virus of IBR. The other properties of this isolate, the type of CPE produced and its sensitivity to ether and chloroform support this identification. These results confirm previous serological evidence about the prevalence of IBR in Saudi Arabia.

The role of the IBR virus in the etiology of the disease syndrome reported here is unknown. Organisms of the genus *Clostridium* were isolated from the same animal from which the IBR virus was isolated (M. Ayaz, Food Technology Section, Regional Agriculture and Water Research Center, Riyadh, personal communication) as well as from other sick and dead animals in the same herd (Y. Sinousi, Veterinary Laboratory, Department of Animal Resources, Ministry of Agriculture and Water, Riyadh, personal communication). Unfortunately, there were no available facilities to fully identify the isolated *Clostridium* organisms. The clinical symptoms might point to a tentative diagnosis of malignant edema and/or enterotoxemia. However, the respiratory and genital signs indicated the involvement of IBR virus. The clostridia are opportunistic pathogens and the lowering of animal resistance due to stress and bad hygienic conditions could be predisposing factors for infection with anaerobic bacteria. Such factors can also play an important role in activating latent infections with IBR virus (Sheffy and Rodman 1973). It is of interest to note that the Zebu and local milking cows housed in the same parlor remained healthy. This might indicate more resistance of these breeds as compared to the Holstein-Friesian cows. In addition, the absence of disease among the dry

Holstein-Friesian cows housed about 200 meters from the affected cattle may be a reflection of less stress and/or better hygienic condition, especially since the same feeds and the same attendants were used for both groups. The pens of the dry cows were open with no crowding of the animals. On the other hand, the cattle in the milking parlor were over crowded and the parlor itself was dirty with very poor ventilation. The syndrome disappeared completely when a multivalent clostridial vaccine was used in this herd and when the hygienic condition of the milking parlor was improved.

Despite our failure to isolate IBR virus from the 2 nasal mucus samples collected from the sick calves, IBR virus was probably involved in the fatal pneumoenteritis syndrome. If samples had been frequently collected from living sick calves as well as during post mortem examination, the possibilities of virus isolation might well have been enhanced.

Respiratory diseases of calves and abortions have been reported on other dairy farms in Saudi Arabia. Because neutralizing antibody against IBR virus was present in serum samples collected from affected animals on those farms, IBR virus has been implicated in the etiology of such syndromes. Accordingly, living attenuated IBR vaccines are routinely used on some dairy farms in Saudi Arabia. The use of this vaccine does not always prevent the appearance of respiratory disease or abortion (M.D. Mackendrick, Veterinarian of SAADCO Dairy Farm, Al-Kharj, personal communication). Therefore, it is necessary to statistically evaluate the disease status on these farms before and after using IBR vaccine. Furthermore, the epizootiological features of infections with IBR virus under Saudi Arabian field conditions require detailed investigation.

Acknowledgment

The authors express their appreciations to Dr. F.A. Ata, Animal Health Research Institute, Cairo, for providing hyperimmune serum against IBR virus. The skillful technical assistance of Mr. N.E. Fadle and Mr. S.A. Abdel-Rahim is acknowledged with thanks.

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(Received 13/02/1984;
in revised form 13/05/1984)

عزل وتصنيف الفيروس المسبب لمرض التهاب الجيوب الأنفية والقصبة الهوائية المعدي بين الأبقار فى المملكة العربية السعودية

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و

ادارة الثروة الحيوانية - وزارة الزراعة والمياه - الرياض - المملكة العربية السعودية

لقد تم عزل الفيروس المسبب لمرض التهاب الجيوب الأنفية والقصبة الهوائية المعدى لأول مرة بالمملكة من عينات مخاط مجموعة من القصبة الهوائية لبقرة نافقة عليها أعراض الإصابة باللاهوائيات. وقد لوحظت التغيرات المرضية على خلايا الزرع النسيجي المحضر من كلية عجل بعد ٤ أيام من الحقن. وقد وجد أن الفيروس المعزول (الرياض/٥) حساس للمعاملة بواسطة الاثير والكلوروفورم وتم معادلته بواسطة سيرم مناعى محضر ضد عتره معروفه لذلك الفيروس. وقد تم مناقشة العلاقة المحتمله بين الإصابه باللاهوائيات وذلك الفيروس - وكذلك استعراض الوضع الوبائى الناتج عن الإصابات بذلك الفيروس في المملكة العربية السعوية.